U.S. Army Public Health Command (Provisional)

USAPHC

TOXICOLOGY STUDY NO. 87-XE-074Zm-09
PROTOCOL NO. 08UG-70-IV09-06-01d
AMES MUTAGENICITY TEST
OF THE RDX REPLACEMENT,
TRIAMINOGUANIDINIUM-1-METHYL-5-
NITRIMINOTETRAZOLATE (TAG-MNT)
MARCH 2010

Approved for public release; distribution unlimited.

Toxicity Tests: 40-5k1

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14. ABSTRACT
The Army EQT Ordnance Environmental Program (OEP) is dedicated to finding replacements for substances causing environmental and/or occupational risks to health. The Army has developed new experimental explosives to be less toxic than RDX yet with similar power and sensitivity. The purpose of this Toxicology Study was to examine the mutagenic potential of TAG-MNT using a novel high throughput Xenometrix MPF™ Ames assay introduced at the USAPHC (Prov) Directorate of Toxicology. TAG-MNT was weakly positive as a mutagen both with and without S9 metabolism only at the highest, limit dose of 2 g/L. TAG-MNT was cytotoxic to S. typhimurium at concentrations at and above 250 mg/L. As TAG-MNT is only weakly mutagenic in the Ames assay, concern about possible carcinogenicity needs to await further genotoxic evaluation. The cytotoxicity of TAG-MNT may be a concern more relevant to its in vivo toxicology. Development of this replacement energetic should continue.

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March 2010

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards. No deviations from the aforementioned regulation affected the quality or integrity of the study or the interpretation of the results.

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EXECUTIVE SUMMARY:
TOXICOLOGY STUDY NO. 87-XE-074Zm-09
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AMES MUTAGENICITY TEST OF THE RDX REPLACEMENT, TRIAMINOGUANIDINIUM-1-METHYL-5-NITRIMINOTETRAZOLATE (TAG-MNT)
MARCH 2010

1. PURPOSE. To provide environmental and occupational health information on new or replacement energetic compounds for Army use in the research, development, testing, and evaluation (RDT&E) of alternatives under the Environmental Quality Technology (EQT) program. This information is necessary for work unit program evaluation.

a. Research, development, testing, training, and use of substances potentially less hazardous to human health and the environment is vital to the readiness of the U.S. Army. Safeguarding the health of Soldiers, civilians, and the environment requires an assessment of alternatives before they are fielded. Continuous assessments begun early in the RDT&E process can save significant time and effort during RDT&E, as well as over the life cycle of the items developed. Residues of pyrotechnics, propellants, explosives, and incendiaries have been found in soil, air, surface, and ground water samples, creating environmental problems and interfering with training activities.

b. The Army EQT Ordnance Environmental Program (OEP) is dedicated to finding replacements for substances causing environmental and/or occupational risks to health. The Army has developed new experimental explosives to be less toxic than RDX yet with similar power and sensitivity. The purpose of this Toxicology Study was to examine the mutagenic potential of TAG-MNT using a novel high throughput Xenometrix MPF™ Ames assay introduced at USAPHC Directorate of Toxicology, and to validate the assay for Good Laboratory Practice compliance (Xenometrix MPF™ is a trademark of Xenometrix AG, Switzerland.)

2. CONCLUSIONS. TAG-MNT was only weakly positive as a mutagen both with and without S9 metabolism at the highest, limit dose of 2 grams per liter (g/L). TAG-MNT has cytotoxic effects at concentrations at and above 250 milligrams per liter (mg/L).

3. RECOMMENDATIONS. As TAG-MNT is only weakly mutagenic in the Ames assay, concern about possible carcinogenicity needs to await further genotoxic evaluation. The cytotoxicity of TAG-MNT may be a concern more relevant to its *in vivo* toxicology. Development of this replacement energetic should continue.

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MARCH 2010

1. REFERENCES. See Appendix A for a listing of references used in this report.

2. PURPOSE. This study was conducted to determine the mutagenicity of Triaminoguanidinium-1-methyl-5-nitriminotetrazolate (TAG-MNT) using the Xenometrix Microplate Format Mutagenicity Assay with five strains of bacteria in compliance with the Organization of Economic Co-operation Development (OECD) Guideline For Testing of Chemicals (OECD, 1997) and U.S. Environmental Protection Agency (USEPA) (Toxic Substance Control Act (TSCA)) Health Effect Testing Guidelines 870.5265 (Title 40 Code of Federal Regulations (CFR) 798.5265).

3. AUTHORITY. This toxicology study addresses, in part, the environmental safety and occupational health (ESOH) requirements outlined in Army Regulations (AR) 200-1 , AR 40-5 , and AR 70-1; Department of Defense Instruction (DODI) 4715.4 (DoDI 4715.4), and Army Environmental Research and Technology Assessment (AERTA, 2007) requirement A (3.3.c), *Compliant Ordnance Lifecycle for Readiness of the Transformation and Objective Forces*. This program is under the direction of the U.S. Army Research, Development and Engineering Command (USA RDECOM) Environmental Acquisition Logistics & Sustainment Program and Environmental Quality Technology (EQT) Pollution Prevention.

4. BACKGROUND.

a. RDX is an important primary explosive necessary in strategic use. The use of RDX at ranges has caused curtailment of range activities because of transport and toxicity issues with ground water contamination. RDX is a known environmental toxicant with an USEPA acute oral minimum risk level (MRL) of 60 micrograms per kilograms per day (µg/kg-day) based on its epileptiform seizure neurotoxicity in humans and rodents (Stone et al., 1969; Burdette et al., 1988; Kasuske et al., 2009; Williams et al., 2010), and a Reference Dose (RfD) of 3 µg/kg-day based on prostatic inflammation in rodents. RDX is also classified as a possible carcinogen (Lish et al., 1984; Parker et al., 2006).

b. The Army EQT Ordnance Environmental Program (OEP) is dedicated to finding replacements for RDX that will reduce or eliminate the health risks from environmental exposure and will reduce adverse ESOH effects; RDX adversely affects the readiness

and costs associated with training (USACHPPM, 2007). By identifying unacceptable ESOH effects early in the acquisition process, unacceptable replacements can be identified and unnecessary budget expenditures can be greatly reduced.

c. In collaboration with U.S. Army Research, Development, and Engineering Command (RDECOM), personnel in the Department of Chemistry and Biochemistry, Ludwig-Maximilian University, Munich, Germany, have synthesized several energetic compounds as possible replacements for RDX. TAG-MNT is one of the lead compounds from this laboratory (Hammer et al., 2005; Klapotke et al., 2008a; Klapotke et al., 2008b).

d. The U.S. Army Public Health Command (Provisional) (USAPHC (Prov)), formerly the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Toxicology Directorate is tasked with providing mutagenicity data for TAG-MNT to determine if it is safe for use by military personnel and civilians living and working near military-training facilities. Therefore, the toxicity data from these studies will help in the recommendations for appropriate exposure guidance.

e. Historically, the mutagenicity of test materials has been evaluated in the agar-plate based Ames assay (Ames et al., 1975). Point Mutations were made in the histidine (*His*) operon in *Salmonella typhimurium*, rendering the bacteria incapable of producing histidine. These mutations are positioned at strategic points within the *His* gene, resulting in *his*-organisms that cannot grow unless histidine is supplied. When a mutagenic event occurs, base substitutions or frameshifts within the *His* gene may cause a reversion to histidine prototrophy. These reverted bacteria will then grow in histidine-deficient media. A chemical's mutagenic potential is assessed by exposing these *his*- organisms to varying concentrations of the chemical and selecting for the reversion event. Medium lacking histidine is used for this selection which allows only those cells that have undergone the reversion to histidine prototrophy to survive and grow. The Ames test for mutagenicity (Ames et al., 1975) is a method widely accepted for evaluation of mutagenic potential by the USEPA (Title 40 CFR 798.5265.), The U.S. Food and Drug Administration (USFDA) and OCED (OECD, 1997).

f. Xenometrix has developed a proprietary microplate format (MPF™) Ames test that provides a convenient, high throughput capability for mutagenicity testing. The test uses the same strains of bacteria required by the USEPA and reduces the assay to a simple, non-agar, 384-well plate methodology that is expedient and cost effective. This report describes the mutagenic effects of TAG-MNT in the MPF™ Ames Assay (Xenometrix MPF™ is a trademark of Xenometrix AG, Switzerland.)

e. The USAPHC (Prov) Good Laboratory Practice (GLP) Policy—Policy Memorandum 74 states:

> *All experiments and studies conducted by any element of the USAPHC Directorate of Toxicology will be compliant with the applicable Good Laboratory Practice (GLP) guidelines reflected in the following regulations:*
>
> *(1). Title 21 Code of Federal Regulations (CFR) Part 58, Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.*
>
> *(2). Title 40 Code of Federal Regulations (CFR) Part 160, Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Good Laboratory Practice Standards: Final Rule.*
>
> *(3). Title 40 Code of Federal Regulations (CFR) Part 792, Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards: Final Rule.* (Memorandum, 9 November 2009,).

f. According to this policy and the fact that the results of these assays may be used in regulatory decisions involving the USEPA or USFDA, these Ames test assays will be conducted in compliance with GLP standards and will follow the appropriate regulatory testing guidelines depending on the test compound (USAPHC (Prov), 2009b).

g. Table 1 identifies the critical events and dates of this study.

Table 1. Critical Study Events

Critical Event	Date of Event
Non-Animal Use Protocol Approved	22 October, 2009
Experimental Start Date	27 October, 2009
Experimental Completion Date	22 February, 2010
Study Completion Date	March 2010

5. MATERIALS

a. Test Substance. The molecular structure of TAG-MNT is shown in Figure 1. TAG-MNT is a light pinkish purple, granular explosive. The material was supplied by RDAR-MEE W, Picatinny Arsenal, Picatinny, NJ, Lot # RDD09A004E001. The compound was certified to be 98-99 percent pure as measured by NMR spectral analysis. The 25-X stock solution of TAG-MNT (50 milligrams per milliliter (mg/mL))

was analyzed via high performance liquid chromatography-UV (CAD Method 98.2) by the Chromatographic Analysis Division (Explosives Team), Directorate of Laboratory Sciences (DLS), USAPHC (Prov). The measured concentration was within 6 percent of theoretical concentration.

Triaminoguanidinium-1-methyl-5-nitriminotetrazolate

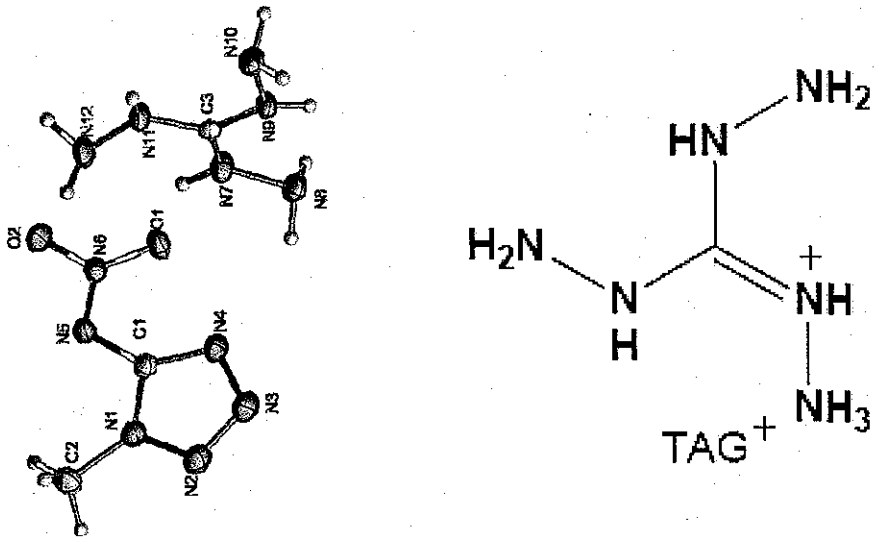

Figure 1. Molecular structure of TAG-MNT

b. Test System. The Xenometrix MPF kit was obtained from ANIARA, Mason, OH. The kit contained a Quality Assurance certificate for each bacterial strain provided in the kit. A list of the kit reagents and supplementary reagents with expiration dates are provided in Appendix B. All reagents were stored in refrigerators and freezers in accordance with directions in the kit as described in Directorate of Toxicology (DTOX) Standing Operating Procedure (SOP) #155 (USAPHC (Prov), 2009a).

c. BacTiter-Glo™ Microbial Cell Viability Assay. This kit was obtained from Promega Corporation, Madison, WI and contains a luminescent reagent that is activated by incubation with ATP in the living bacteria. (BacTiter-Glo™ is a trademark of Promega Corporation Madison, WI.)

d. Quality Assurance. The USPHC (Prov) Quality Systems Office audited critical phases of this study. Appendix B provides the dates of these audits along with the audited phases and the dates that the results of the audits were reported to Management and the Study Director.

e. Study Personnel. Appendix C contains the names of persons contributing to the performance of this study.

6. METHODS.

a. The experimental design and general procedures of this study were conducted under the USAPHC (Prov) DTOX SOP for the Xenometrix MPF Ames Test Kit (USAPHC (Prov), 2009a). The test kit is designed to determine the mutagenicity of a test material in compliance with the USEPA and OECD Guidelines for the testing of chemicals 471 Bacterial Reverse Mutation Test (OECD, 1997) and USAPHC (Prov) Type Protocol: The Ames Test For Mutagenicity # 08UG-70-iv09-06-01 (USAPHC (Prov), 2009b).

b. Preliminary experiments demonstrated that TAG-MNT is readily soluble in water up to 100 mg/mL at room temperature. For the Ames Assay, the limit concentration of the test material is considered to be 2 grams per liter (g/L) or 2 mg/mL. Per DTOX SOP 155 (USAPHC (Prov), 2009b), a 25X stock solution of TAG-MNT was prepared in sterile water at 50 mg/mL.

c. For any given experiment, TAG-MNT was tested against a single bacterial strain, i.e., there were five experiments, one for each of the five bacterial strains. See Appendix E for a detailed description of the assay procedure. Typically, the bacteria strain of choice was thawed according to DTOX SOP #155 (USAPHC (Prov), 2009a) and expanded in Growth Media with shaking at approximately 37 degrees Celsius (°C) overnight with or without ampicillin as appropriate for the specific strain. After validation of sufficient bacterial density with a spectrophotometer, the bacterial suspension was diluted with Incubation Medium and aliquoted to tissue culture wells containing serial dilutions of 25X concentrates of the test material. The suspensions were incubated with shaking for 90 min at approximately 37 °C with and without the inclusion of S9 liver extract (±S9); inclusion of S9 determines if a mutagen is generated from the parent compound due to metabolism by liver enzymes. At the end of the incubation, each well was diluted 11-fold with purple Indicator Medium and 50 microliters (µL) aliquots distributed appropriately into 384-well plates. These plates were incubated anaerobically for 2 days at approximately 37 °C and then scored for presence of revertant colonies, i.e., evidence of a positive mutagenic event (Appendix E). Positive wells are those that have turned yellow or have a bacterial colony visible on the bottom of the well. Any indication of a color change from purple to yellow is included in the positive count. The indicator turns from purple to yellow due to a pH change resulting from metabolism of living, mutated bacteria.

d. Criteria for evaluation and interpretation of mutagenicity

(1) A positive control appropriate for each strain was run coincidently with the test material to assure the assay was valid for each run. The assay as a whole is determined to be valid if the number of control background reversions is within prescribed limits and the number of positive control reversions is within prescribed limits (USAPHC (Prov), 2009a; USAPHC (Prov), 2009b).

(2) There are several criteria for determining a positive result. The number of reversions induced by the test compound is at least 2-fold above the background control, and there is a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation system. Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results. However, statistical significance should not be the only determining factor for a positive response. A test substance for which the results do not meet the above criteria is considered nonmutagenic in this test (Xenometrix, 2009).

(3) Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgment about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated.

(4) Positive results from the bacterial reverse mutation test indicate that a substance induces point mutations by base substitutions and/or frame shifts in the genome of either *Salmonella typhimurium* and/or *Escherichia coli (E. coli)*. Negative results indicate that under the test conditions, the test substance is not mutagenic in the tested species.

e. Coincident with the mutagenic incubation, a duplicate plate was prepared for determination of cytotoxicity of the test material using ATP luminescence. After the 90 min incubation at approximately 37 °C, samples from the incubation plate were aliquoted to a 96-well plate. An equal volume of luminescent reagent was added to each well according the method described for the BacTiter-Glo Microbial Cell Viability Assay.

7. RESULTS.

a. Toxicity of TAG-MNT Figure 2 illustrates the levels of toxicity demonstrated by TAG-MNT. The level of toxicity depended on the strain of bacteria, but evidenced toxicity at concentrations of 250 µg/mL, 250 mg/L and above.

b. Mutagencity of TAG-MNT The Raw data for the mutagenicity tests and calculations are provided in Appendix C. The results are summarized in Table 3. TAG-MNT was mutagenic at the limit dose of 2 g/L in three of the five strains both with and without S9 incubation. TAG-MNT was not mutagenic in the TA1537 or *E. coli* strains.

c. Criteria for Valid Assay. In all Ames assays conducted for TAG-MNT, (with the exception of the first *E. coli* – S9 assay which was repeated) the background control and the positive controls for incubations ± S9 were within the limits specified by SOP #155 (USAPHC (Prov), 2009a), i.e., all assays met the criteria for validity. In most cases, TAG-MNT was mutagenic only at the limit dose of 2 mg/mL and showed a true dose response only in TA100 strain –S9. However, because a positive result was seen in multiple cases at 2 mg/mL, the weight of evidence indicates that TAG-MNT is mutagenic. Cytotoxicity of TAG-MNT in the BacTiter-Glo assay is indicated when the luminosity of ATP in compound-treated cultures is decreased below the levels in vehicle-treated cultures; the level of ATP generated luminsoty correlates with the number of living bacteria. The four strains tested demonstrated differential sensitivity to TAG-MNT: TAG-MNT was cytotoxic at 1000 µg/mL in the TA1535 and E. coli strains; cytotoxic at 500 µg/mL in the TA98 strain and at 250 µg/mL in the TA 100 strain. However, compounds can still be mutagenic even at cytotoxic concentrations (Xenometrix, 2009).

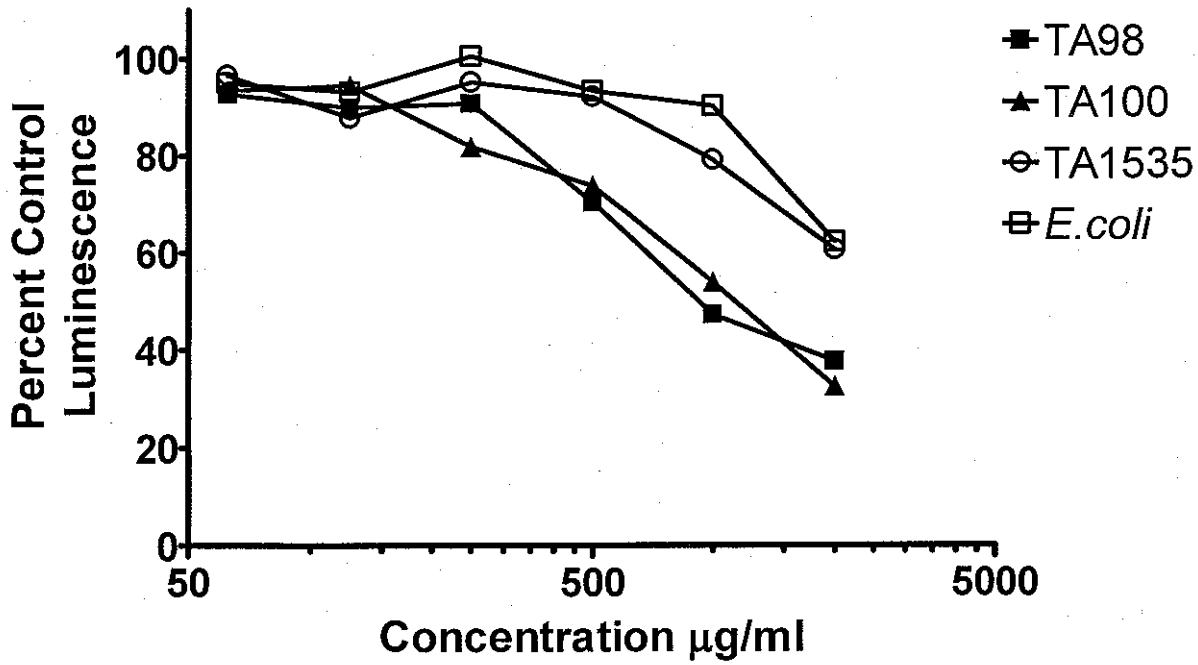

Figure 2. Cytotoxicity of TAG-MNT

Table 2. Mutagenicity of TAG-MNT

Strain	TAG-MNT	
	-S9	+S9
TA98	+ 2 mg/mL	+ 2 mg/mL
TA100	+ 1 mg/mL	negative
TA1535	+ 2 mg/mL	+ 2 mg/mL
TA1537	negative	negative
E. coli	negative	negative

8. DISCUSSION.

a. TAG-MNT was weakly positive as a mutagen both with and without S9 metabolism at the highest, limit dose. Although meeting the criteria for validity as a mutagen, the number of mutant reversions was less than 3.5-fold above background. The positive controls for the *salmonella* strains were greater than 10-fold above background.

b. TAG-MNT evidenced cytotoxicity at concentrations at and above 250 mg/mL. This was observed directly in the BacTiter-Glo assay as a reduction in ATP-induced luminescence, and indirectly in the Xenometrix MPF as a reduction in the number of mutant reversion below background, e.g., raw data for TA1537 +S9. TAG-MNT was also found to be toxic in the 48 hr neutral red uptake human liver cell line assay with an inhibitory concentration (IC_{50}) of 316 µg/mL (USACHPPM, 2008). Although TAG-MNT can be toxic, it did not kill all bacteria at the concentrations tested, i.e. there is still ATP signal in the BacTiter-Glo assay at the limit dose (Fig. 2). TAG-MNT is apparently mutagenic to the surviving bacteria as the number of mutant reversions meet the criteria for validity.

c. There are significant procedural differences between the traditional agar plate Ames test and the Xenometrix MPF Ames Test. In the MPF Ames Test, bacteria are exposed to the compound for 90 minutes before 11-fold dilution with Indicator medium and 2-day incubation for evidence of mutation. In the agar plate assay, compound is included in a uniform 2.7 mL agar overlay onto a bottom agar layer of bacteria and exposure is 72 hours. Data from agar plate Ames test is reported in terms of the

amount of test compound present in the agar plate (i.e., µg/plate). However, because of the uniformity of the agar plate assay (i.e., a 2.7-mL overlay), µg/plate can be readily converted into µg/mL, the unit of concentration used in the Xenometrix MPF Ames Test (Kenyon et al., 2007).

8. CONCLUSIONS. TAG-MNT was weakly positive as a mutagen both with and without S9 metabolism at the highest, limit dose of 2 g/L. TAG-MNT has cytotoxic effects at concentrations at and above 250 mg/L.

9. RECOMENDATIONS. As TAG-MNT is only weakly mutagenic in the Ames assay, concern about possible carcinogenicity needs to await further geneotoxic evaluation. Development of this replacement energetic should continue.

APPENDIX A

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APPENDIX B

QUALITY ASSURANCE STATEMENT

For: DTOX Toxicology Study No. 87-XE-074Zm-09, Protocol No. 08UG-70-IV09-06-01d. "Ames Mutagenicity Test of the RDX replacement, Triaminoguanidinium-1-Methyl-5-Nitriminotetrazolate (TAG-MNT)", the following critical phases were inspected/audited by the Quality Systems Office (QSO):

Critical Phase Inspected/Audited (QSO Checklist #)	Date Inspected /Audited	Date Reported to Management/SD
Type Protocol Review (QSO checklist # 1.26)	10/23/2009	11/05/2009

Critical Phase Inspected/Audited (QSO Checklist #)	Date Inspected /Audited	Date Reported to Management/SD
Maintenance and Calibration of Equipment	10/29/2009	11/05/2009
Compliance with Study Protocol	10/30/2009	11/16/2009
Reagents and Solutions	10/30/2009	11/16/2009
Test Article Characterization	10/30/2009	11/16/2009
Maintenance and Calibration of Equipment	01/12/2010	01/22/2010
AMES Test - Assay Procedures - Day 2	01/12/2010	01/22/2010
Raw Data Documentation Procedures	01/14/2010	02/01/2010
AMES Test - Assay Procedures - Day 4	01/14/2010	01/22/2010
Excel Spreadsheet Verification	01/26/2010	02/01/2010
Test Culture Growth Determination	01/26/2010	02/01/2010
Cell Viability Assay	02/02/2010	02/22/2010
Dosing Solution Verification	02/12/2010	02/16/2010
Test System Storage Conditions	02/23/2010	03/01/2010
Reagent Storage Conditions	02/23/2010	03/01/2010
Final Study Report Review	03/24/2010	03/25/2010
Study Raw Data Review	03/24/2010	03/25/2010

Note: All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.

Michael P. Kefauver
GLP Assessor, QSO

03/26/10
Date

Gene Sinar
Team Leader, QSO

3/26/2010
Date

APPENDIX C

ARCHIVES AND STUDY PERSONNEL

C-1. ARCHIVES

a. All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in the storage facilities of the Toxicology Directorate, USAPHC (Prov), for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

b. Records on the test system will be archived by the Toxicology Directorate, for a minimum of five (5) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

c. The present study used the laboratory project number: 87-XE-074Z-09, toxicology study number: 87-XE-074Zm-09, and protocol number 08UG-70-iv09-06-01d for all filings.

d. The protocol, raw data, summary data, and the final report pertaining to this study will be physically maintained within Building E-2100, USAPHC (Prov). These data may be scanned to a computer disk. Scanned study files will be stored electronically in Room 3010, Building E-2100, USAPHC (Prov), APG, MD, 21010.

e. Archived SOPs and maintenance and calibration logbooks may be found in Room 1026, Building E-2100, USAPHC (Prov), APG, MD, 21010.

g. Archivist: Kristin T. Newkirk

C-2. PERSONNEL.

a. Management: Glen Leach, Acting Director of Toxicology; Mark Johnson, Ph.D., Program Manager, Health Effects Research Program (HERP)

b. Study Director: Larry Williams, Biologist, HERP.

c. Quality Assurance: Michael P. Kefauver, Chemist, Quality Systems Office

APPENDIX D

AMES TEST REAGENTS

Ames Reagents	Source	Lot #	Date Expiration
AG-TA98 *hisD3052 Salmonella typhimurium*	Xenometrix	03	9/1/2010
AG-TA100 *hisG46 Salmonella typhimurium*	Xenometrix	03	11/1/2010
AG-TA1535 his G46 *Salmonella typhimurium*	Xenometrix	01	11/1/2009
AG-TA1537 hisC3076 *Salmonella typhimurium*	Xenometrix	01	11/1/2009
E. coli wp2 [pKM101] trpE65 Escherichia coli	Xenometrix	P02	5/1/2010
E. coli wp2 *uvrA trpE65 Escherichia coli*	Xenometrix	U02	3/1/2010
Ampicillin	Xenometrix	083	10/1/2009
MOLTOX™ S-9 Liver Mitochondrial Supernatant	Xenometrix	2361	11/7/2010
N4-aminocytidine	Xenometrix	060H09241	2/20/2011
2-nitrofluorene	Xenometrix	2208NF	4/1/2010
4-nitroquinoline-N-oxide	Xenometrix	128NQ	6/1/2010
9-aminoacridine	Xenometrix	1004AAHC	4/1/2010
Ames MPF Exposure Medium	Xenometrix	C06825-1P	6/1/2010
Ames MPF Reversion Indicator Medium	Xenometrix	C06850-1P	6/1/2010
Ames MPF Growth Medium	Xenometrix	C06851-1P	6/1/2010
E.Coli Reversion Indicator Medium	Xenometrix	C11145P	11/1/2010
E.Coli Exposure Medium	Xenometrix	C04759P	4/1/2010
KCl	Sigma-Aldrich	NA	3/1/2011
$MgCl_2x6H_2O$	Sigma-Aldrich	NA	3/1/2011
Glucose-6-phosphate	Sigma-Aldrich	NA	3/1/2011
NADP	Sigma-Aldrich	NA	3/1/2011
NaH_2PO_4	Sigma-Aldrich	NA	3/1/2011

APPENDIX E
AMES TEST RAW DATA AND CALCULATIONS

TA98

Compoun	TAG-MNT			10/29/2009	
TA 98 -S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 98 -S9
62.5	1	1	1		1
125	0	1	1		0
250	0	2	0		0
500	3	1	1		1
1000	1	1	1		0
2000	3	4	2		0
Pos. Control	36	46			

Compoun	TAG-MNT			10/29/2009	
TA 98 +S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 98 +S9
62.5	3	2	3		1
125	2	0	3		1
250	4	0	3		2
500	2	2	2		2
1000	1	5	2		1
2000	5	6	2		1
Pos. Control	48	48			

TAG-MNT

TA 98 -S9						Assay Date:		10/29/2009	
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	0.33	1.00	0.52	1.00				
62.5	3	1.00		0.00		1.00	1.00	0.0338	
125	3	0.67		0.58		0.67	0.67	0.2035	
250	3	0.67		1.15		0.67	0.67	0.2763	
500	3	1.67		1.15		1.67	1.67	0.0207	
1000	3	1.00		0.00		1.00	1.00	0.0338	
2000	3	3.00		1.00		3.00	3.00	0.0005	
Pos. Contro	2	41.00		7.07					

TAG-MNT

TA 98 +S9						Assay Date:		10/29/2009	
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	1.33		0.52	1.85				
62.5	3	2.67		0.58		2.00	1.44	0.0048	
125	3	1.67		1.53		1.25	0.90	0.3131	
250	3	2.33		2.08		1.75	1.26	0.1377	
500	3	2.00		0.00		1.50	1.08	0.0338	
1000	3	2.67		2.08		2.00	1.44	0.0793	
2000	3	4.33		2.08		3.25	2.34	0.0047	
Pos. Contro	2	48.00		0.00					

TA100

Compoun	TAG-MNT			11/2/2009	
TA 100 -S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 100 -S9
62.5	4	9	14		2
125	9	8	3		3
250	14	14	6		7
500	4	11	7		6
1000	13	15	13		5
2000	19	17	30		4
Pos. Control	48	48	48		

Compoun	TAG-MNT			11/2/2009	
TA 100 +S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 100 +S9
62.5	0	2	0		5
125	0	0	0		1
250	1	0	0		3
500	1	1	0		1
1000	0	0	0		1
2000	2	1	1		0
Pos. Control			35		

TAG-MNT

TA 100 -S9						Assay Date:		11/2/2009	
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	4.50		1.87	6.37				
62.5	3	9.00		5.00		2.00	1.41	0.0398	
125	3	6.67		3.21		1.48	1.05	0.1154	
250	3	11.33		4.62		2.52	1.78	0.0066	
500	3	7.33		3.51		1.63	1.15	0.0733	
1000	3	13.67		1.15		3.04	2.15	0.0001	
2000	3	22.00		7.00		4.89	3.45	0.0002	
Pos. Contro	3	48.00		0.00					

TAG-MNT

TA 100 +S9						Assay Date:		11/2/2009	
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	1.83		1.83	3.67				
62.5	3	0.67		1.15		0.36	0.18	0.1779	
125	3	0.00		0.00		0.00	0.00	0.0692	
250	3	0.33		0.58		0.18	0.09	0.1108	
500	3	0.67		0.58		0.36	0.18	0.1657	
1000	3	0.00		0.00		0.00	0.00	0.0692	
2000	3	1.33		0.58		0.73	0.36	0.3341	
Pos. Contro	1	35.00							

TA1535

Compoun	TAG-MNT			1/25/2010	
TA 1535 -S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 1535 -S9
62.5	2	0	1		2
125	2	3	4		1
250	4	5	5		0
500	1	1	2		1
1000	2	2	3		1
2000	6	4	9		3
Pos. Control	48	48	48		

Compoun	TAG-MNT			1/25/2010	
TA 1535 +S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 1535 +S9
62.5	0	0	0		1
125	0	1	1		0
250	1	2	3		0
500	1	2	2		1
1000	3	2	1		1
2000	3	8	5		2
Pos. Control	25	29	23		

TAG-MNT

TA 1535 -S9 — Assay Date: 1/25/2010

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	6	1.33		1.03	2.37			
62.5	3	1.00		1.00		0.75	0.42	0.3295
125	3	3.00		1.00		2.25	1.27	0.0274
250	3	4.67		0.58		3.50	1.97	0.0007
500	3	1.33		0.58		1.00	0.56	0.5000
1000	3	2.33		0.58		1.75	0.99	0.0852
2000	3	6.33		2.52		4.75	2.68	0.0016
Pos. Contro	3	48.00		0.00				

TAG-MNT

TA 1535 +S9 — Assay Date: 1/25/2010

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	6	0.83	1.00	0.75	1.59			
62.5	3	0.00		0.00		0.00	0.00	0.0532
125	3	0.67		0.58		0.67	0.42	0.3743
250	3	2.00		1.00		2.00	1.26	0.0437
500	3	1.67		0.58		1.67	1.05	0.0698
1000	3	2.00		1.00		2.00	1.26	0.0437
2000	3	5.33		2.52		5.33	3.36	0.0018
Pos. Contro	3	25.67		3.06				

TA1537

Compoun	TAG-MNT			2/1/2010	
TA 1537 -S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 1537 -S9
62.5	1	0	1		1
125	0	0	0		0
250	1	0	0		0
500	0	0	1		0
1000	0	0	0		0
2000	0	0	1		1
Pos. Control	48	48	48		

Compoun	TAG-MNT			2/1/2010	
TA 1537 +S9					Spontaneous
Conc. (µg/ml)	Replicat e #1	Replicat e #2	Replicat e #3		TA 1537 +S9
62.5	1	1	1		3
125	4	0	3		3
250	3	2	1		2
500	1	1	1		1
1000	2	2	1		2
2000	1	1	1		2
Pos. Control	4	25	20		

TAG-MNT

TA 1537 -S9 — Assay Date: 2/1/2010

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)
0	6	0.33	1.00	0.52	1.00			
62.5	3	0.67		0.58		0.67	0.67	0.2035
125	3	0.00		0.00		0.00	0.00	0.1579
250	3	0.33		0.58		0.33	0.33	0.5000
500	3	0.33		0.58		0.33	0.33	0.5000
1000	3	0.00		0.00		0.00	0.00	0.1579
2000	3	0.33		0.58		0.33	0.33	0.5000
Pos. Contro	3	48.00		0.00				

TAG-MNT

TA 1537 +S9 — Assay Date: 2/1/2010

Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	2.17		0.75	2.92				
62.5	3	1.00		0.00		0.46	0.34	0.0179	Cytotoxic
125	3	2.33		2.08		1.08	0.80	0.4297	
250	3	2.00		1.00		0.92	0.69	0.3924	
500	3	1.00		0.00		0.46	0.34	0.0179	Cytotoxic
1000	3	1.67		0.58		0.77	0.57	0.1753	
2000	3	1.00		0.00		0.46	0.34	0.0179	Cytotoxic
Pos. Contro	3	16.33		10.97					

E Coli Combo

Compoun	TAG-MNT			1/11/2010	
EC Combo -S9					Spontaneous
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3		EC Combo -S9
62.5	5	4	3		2
125	6	3	3		6
250	4	2	3		6
500	4	3	5		4
1000	2	2	8		3
2000	0	4	4		9
Pos. Control	5	2	7		

Compoun	TAG-MNT			1/11/2010	
EC Combo +S9					Spontaneous
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3		EC Combo +S9
62.5	5	4	2		4
125	7	5	9		5
250	4	4	6		1
500	10	8	3		9
1000	2	7	6		6
2000	4	3	9		3
Pos. Control	15	15	11		

Compoun	TAG-MNT			2/22/2010	
EC Combo -S9					Spontaneous
Conc. (µg/ml)	Replicate #1	Replicate #2	Replicate #3		EC Combo -S9
62.5	1	4	2		2
125	5	4	2		5
250	1	2	2		3
500	0	5	4		1
1000	3	0	3		2
2000	1	2	1		5
Pos. Control	19	22	25		

TAG-MNT						Assay Date:	1/11/2020		
EC Combo -S9									
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	5.00		2.53	7.53				
62.5	3	4.00		1.00		0.80	0.53	0.2708	
125	3	4.00		1.73		0.80	0.53	0.2815	
250	3	3.00		1.00		0.60	0.40	0.1201	
500	3	4.00		1.00		0.80	0.53	0.2708	
1000	3	4.00		3.46		0.80	0.53	0.3162	
2000	3	2.67		2.31		0.53	0.35	0.1116	
Pos. Contrc	3	4.67		2.52					

TAG-MNT						Assay Date:	1/11/2020		
EC Combo +S9									
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	4.67		2.73	7.40				
62.5	3	3.67		1.53		0.79	0.50	0.2909	
125	3	7.00		2.00		1.50	0.95	0.1179	
250	3	4.67		1.15		1.00	0.63	0.5000	
500	3	7.00		3.61		1.50	0.95	0.1545	
1000	3	5.00		2.65		1.07	0.68	0.4334	
2000	3	5.33		3.21		1.14	0.72	0.3764	
Pos. Contrc	3	13.67		2.31					

TAG-MNT						Assay Date:	2/22/2020		
EC Combo -S9									
Conc. (µg/ml)	n	mean # pos. Wells	Corr. mean	SD	Base-line	Fold increase (over zero value)	Fold increase (over baseline)	t-test p-value (unpaired, 1-sided)	
0	6	3.00		1.67	4.67				
62.5	3	2.33		1.53		0.78	0.50	0.2909	
125	3	3.67		1.53		1.22	0.78	0.2909	
250	3	1.67		0.58		0.56	0.36	0.1170	
500	3	3.00		2.65		1.00	0.64	0.5000	
1000	3	2.00		1.73		0.67	0.43	0.2152	
2000	3	1.33		0.58		0.44	0.29	0.0737	
Pos. Contrc	3	22.00		3.00					